

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Needleman et al.)
) Attorney Docket
 Serial No.: 08/785,997) 6221/68346
) MON-101.0
 Filed: January 21, 1997)
) Art Group:
 For: AN IMMUNOLOGICAL PROCESS) 1642
 FOR INCREASING THE HDL)
 CHOLESTEROL CONCENTRATION)
 Examiner: T. Scheiner)

DECLARATION OF PHILIP NEEDLEMAN AND
KEVIN GLENN PURSUANT TO 37 C.F.R. § 1.131

Assistant Commissioner for Patents
 Washington, D.C. 20231

Sir:

PHILIP NEEDLEMAN, Ph.D. and KEVIN GLENN, Ph.D.

Declare:

1. That they are the Philip Needleman and Kevin Glenn
 that are the named inventors of the subject patent application;

2. That they are employed by G.D. Searle, Co.,
 (Searle) a wholly owned subsidiary of the Monsanto Company
 (Monsanto), the assignee of the above-identified patent
 application;

3. That Dr. Needleman holds the positions of Senior
 Vice President of Research and Development at Searle and Chief

*Considered
 HE
 9/12/99*

3. That Dr. Needleman holds the positions of Senior Vice President of Research and Development at Searle and Chief Scientist at Monsanto, whereas Dr. Glenn holds the titles of Fellow and Group Leader of Atherosclerosis Research at Searle;

4. That Elaine Krul, Ph.D., who is presently employed by the Nutrition and Consumer Sector, a division of the Monsanto Company, was formerly employed by Searle between February of 1994 and February of 1998 doing laboratory research related to atherosclerosis;

5. That during her employment by Searle, she carried out her research under the direction and control of one or both of Drs. Needleman and Glenn;

6. That the work described in this Declaration was carried out by Dr. Krul or a person under her direction and control, and was carried out in the State of Missouri, the United States of America;

7. That the work described in this Declaration was completed on a date prior to November 7, 1996, the date of publication of WO 996/34888;

8. That the documents attached to this Declaration are true copies of one or more of Dr. Krul's laboratory

notebooks and an invoice, except that the dates and notebook numbers have been obscured for the purposes of this Declaration;

9. That collective Exhibit 1 contains pages 158, 159 and 160 from one of her notebooks (notebook A) that contains the amino acid residue sequence of cholesteryl ester transfer protein (CETP), using single letter code, in which peptide sequences corresponding to RABCTP-2, RABCTP-3, RABCTP-4, RABCTP-5, RABCTP-6, and RABCTP-7 are underlined and identified as such;

10. That those peptides of Paragraph 9 correspond to SEQ ID Nos: 2, 3, 4, 5, 6, and 7, respectively of the above-identified application;

11. That enclosed Exhibit 2 is a copy of page 161 of that same notebook that reiterates the names of those polypeptides and provides a schematic of a branched oligolysine antigenic carrier molecule referred to a Multiple Antigenic Peptide Backbone that when conjugated to a polypeptide is also referred to as MAP in her notebooks, and is noted at page 18, lines 11-19 of the above-identified application;

12. That enclosed Exhibit 3 is a copy of an invoice from Genosys Biotechnologies, Inc., of The Woodlands, Texas (Genosys) directed to Dr. Krul for conjugates of MAP-covalently-bonded polypeptides RABCTP-2, -3, -4, -5, -6 and -7;

13. That twelve rabbits were immunized on two occasions, two each, with a MAP-linked polypeptide conjugate received from Genosys;

14. That those two immunizations were recorded on pages 165, 166 and 167 of her above notebook, copies of which are enclosed as collective Exhibit 4;

15. That the samples of the immunized rabbits' blood were taken thereafter and were assayed for total cholesterol (TC) and high density lipoprotein (HDL-C);

16. That enclosed Exhibit 5 is a copy of page .071 of a second of her notebooks (notebook B) that contains graphs of the total cholesterol (TC) and high density lipoprotein (HDL-C) for each of the rabbits used in the study from prior to the immunizations (day zero) through at least 75 days thereafter, and in which the boxes within each graph identify each peptide with a shortened designation (CTP-number rather than RABCTP-number) because of space considerations;

17. That those data indicate a general lowering of both total cholesterol and high density lipoprotein for each of the peptides used;

18. That total cholesterol was determined in a standard laboratory assay commercially available from Wako Pure

Chemical Industries, Inc. adapted to a 96-well microtiter plate format;

19. That high density lipoprotein was determined in a standard laboratory assay using a commercially available kit from Sigma Chemicals, Inc., of St. Louis, MO;

20. That enclosed Exhibit 6 is a copy of page 072 of her notebook B showing data for rabbits immunized with conjugates of free polypeptides RABCTP-2, -3, -4, -6 and -7 (again referred to as CTP-2, CTP-3 etc.), also obtained from Genosys, that were individually covalently bonded to tuberculin purified protein derivative (PPD) to form conjugates, and prepared as discussed in Example 1B of the above-identified application, beginning at page 37;

21. That the results shown in Exhibit 6 were obtained following two immunizations with the conjugates using the assay procedures discussed in relation to Exhibit 5;

22. That all statements made herein of his (their) own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the

Serial No.: 08/785,997

-6-

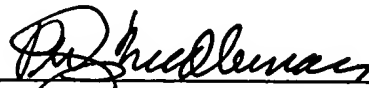
United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Enclosures

Exhibits 1-6

2/1/99

Date



Philip Needleman

6 - January, 1999

Date



Kevin Glenn

Number	Subject	Project Number
160	Rabbit anti-CETP Project Choice of Peptides for Immunization	SEARLE

RABBIT CETP SEQUENCE - RABCTP-5 / RABCTP-6 / RABCTP-7

RABCTP-7

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQ¹RAGY²PDVSGERAVM³

10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNSVVF⁴KGTLNYS

60 70 80 90 100

YTSAWGLQINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110 120 130 140 150

RABCTP-6

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMAD⁵FVQT

160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARA⁶AFQEGRLVLSLTGDEFKKVLETQG

260 270 280 290 300

EDTNQE⁷IFQELSRGLPTGQAQVAVHCLKVPKISCQNRGVVSSSVAVTFR

310 320 330 340 350

RABCTP-5

FPRPDGREAVAYRFEEDIITTVQASYSOKKLEFLHLLDEFCVPASGRAGSS

360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSES⁸LQSSLRSLIATVG⁹IPEVMSRLEVAFT

410 420 430 440 450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHL¹⁰LVDFLQSL¹¹S

460 470 480 490

RABBIT CETP SEQUENCE - RABCTP-3 / RABCTP-4

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAQFQAGYPDVSGERAVM
10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVFKGTLNYS
60 70 80 90 100

YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL
110 120 130 140 150

LLHLQGEREPGWLKQLETFNFI SFTLKLILKGQVCNEINTISNIMADFVQT
160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF
210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARAQFQGRVLVSLTGDEFKKVLETQG
260 270 280 290 300

FDTNQEIQELSRGLPTGQAQVAHVCLKVPKISQNRGVVVSSSVAVTFR
310 320 330 340 350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS
360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSESLQSSLRSLIATVGIPVMSRLEVAFT
410 420 430 440 450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDFLQSL
460 470 480 490

RABCTP-4

RABCTP-3

RABBIT CETP SEQUENCE - RABCTP-2

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQIRAGYPDVSGERAVM

10	20	30	40	50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

60	70	80	90	100

YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110	120	130	140	150

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

160	170	180	190	200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210	220	230	240	250

PPGLLGDSRMLYFWFSDQVLNSLARAQFQEGRLVLSLTGDEFKKVLETQG

260	270	280	290	300

FDTNQEIFQELSRGLPTGQAQVAVHCLKVPKISCQNRGVVSSSVAVTFR

310	320	330	340	350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS

360	370	380	390	400

ANLSVALRTEAKAVSNLTESRSESLOSSLRSLIATVGIPVMSRLEVAFT

410	420	430	440	450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDFLQSL

460	470	480	490

Project Number	Subject	Book Number
SEARLE	Rabbit anti-CETP Project Choice of Peptides for Immunization	Page 161

Summary of Peptide Sequences Submitted to Genosys to couple on MAP:

RABCTP-2	306-325	(#001)	Order # 054215 P.O.# B002455
RABCTP-3	345-364	(#002)	40µm of each Made - Immuno
RABCTP-4	475-496	(#003)	Grade (>60% pure)
RABCTP-5	370-389	(#004)	<u>Made on MAPS Resin:</u> See reference: Butz, S. et al. (1994) Peptide Research 7: 20-23.
RABCTP-6	150-169	(#005)	
RABCTP-7	42-61	(#006)	

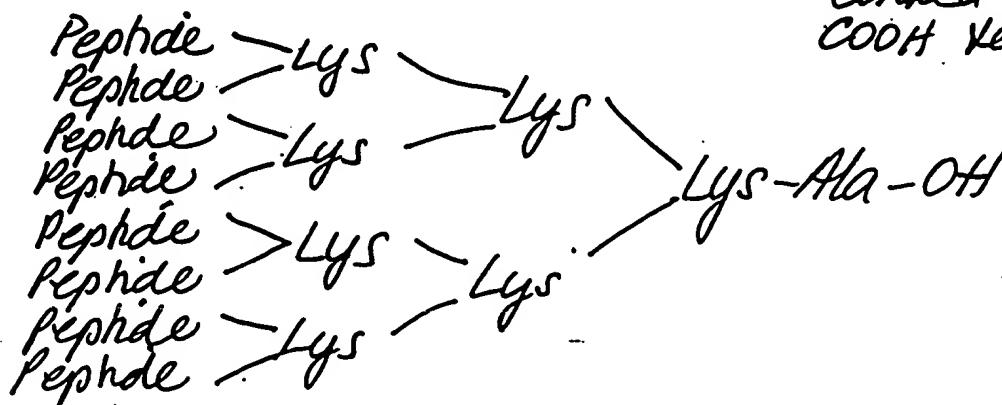
Decided to try coupling to Multiple Antigenic Peptide Backbone as a way to avoid "carrier" protein which will generate a lot of antibodies of and in itself.

One of the original references to this method is:

Posnett, DN, ~~McGrath~~ McGrath H, & Tam JP (1988)
J. Biol. Chem. 263: 1719-1725.

Structure of MAP:

NOTE: Peptides are linked via their COOH termini!!



Author's Signature

Elaine Kuel

Read and Understood By

Jan

GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Order No.

Page 2

ORDER

54215

Bill To
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.
ST. LOUIS MO 63167

ST. LOUIS MO 63167

---CONTACT---

BARB GRIFFARD
314-694-1000 X 6825

---CONTACT---

314-694-4218

Customer: 100 6316701 KRUI
Salesman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
P/O Number: B002455

ORDERED:

SHIPPED:

INVOICED:

LINE	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
5.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-6 LLL HLG GER EPG HLX QLF TH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	
<p>MW 2393 Lot P157A-030 Peptide 54215-005 6.000 1.00 0.00 PEPIG/40</p>									
6.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-7 DVS GER AVH LLG RVK YGL HH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	
<p>MW 2215 Lot P157A-032 Peptide 54215-006</p>									

COMMENTS:

cleaned by 95% TFA, ether extracted 3X, suspended in
6 M TFA/H₂O - 1-2% acetic acid reagent
+ lyophilized

SALES TAX

MISCELLANEOUS

FREIGHT

4.624.00

TOTAL

PACKING LIST

GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

1-800-234-5200
Stacey ext #127
Hogue

[615-343-1465]
James Tam

0.37mm/g
0.37µm/mg term

Dr. Stanfield

Page 1

713-363-3693 ext 13

Order No.

ORDER

54215

Bill To
MONSANTO COMPANY
ACCOUNTS PAYABLE. N2F
800 NORTH LINDBERGH BLVD.

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD
ST. LOUIS MO 63167

ST. LOUIS MO 63167

CONTACT
BARB GRIFFARD
314-694-1000 X 6825

CONTACT
314-694-4218

Customer: 100 6316701 KRU1
Salesman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
/O Number: B002455

ORDERED:

SHIPPED:

INVOICED:

THE	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
1.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 UM IMMUHO GRADE RABCTP-2 EIF QEL SRG LPT GQA QVA VH RESIDUES IMMUHO GRADE MAPS RESIN	20.00	35.00			
				MW 2181 Lot P157A-022 peptide # 54215-001					
2.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 UM IMMUHO GRADE RABCTP-3 VAV TFR FPR PDG REA VAY RF RESIDUES IMMUHO GRADE MAPS RESIN	20.00	35.00			
				MW 2355 Lot P157A-024 peptide # 54215-002					
3.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 UM IMMUHO GRADE RABCTP-4 LLL QMD FGF PKH LLV DFL QSL S RESIDUES IMMUHO GRADE MAPS RESIN PEP SYN	22.00	35.00			
				MW 2562 Lot # P157A-026 peptide # 54215-003					
4.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 UM IMMUHO GRADE RABCTP-5 TTY QAS YSQ KXL FLH LLD FQ RESIDUES IMMUHO GRADE MAPS RESIN	20.00	35.00			
				MW 2368 Lot P157A-028 peptide # 54215-004					

ACKING LIST

T I N U E

Project Number:	Subject: <i>Rabbit anti-CETP Project</i>	Book Number
SEARLE	<i>Immunization Procedure</i>	Page 165

Immunization of Rabbits

Subcutaneously administered (0.05-0.1 ml/site)
along rabbit's back - done by Margi Baldwin.

Rabbits in Room 105 (Tattooed)

#1, #2	RABCTP-2	(3.3mg total ÷ 2 rabbits))
#3, #4	RABCTP-3	(3.4mg "))
#5, #6	RABCTP-4	(3.2mg "))
#7, #8	RABCTP-5	(3.2mg "))
#9, #10	RABCTP-6	(3.3mg "))
#11, #12	RABCTP-7	(3.5mg "))

INVESTIGATOR: _____ DATE: _____
 room #: _____ phone # _____ Mail Zone: Tam
 Technician: _____

RE-
immune
era
vlected

red
pen in
ignob
-20°C.

Animal ID #	Blood	Serum/Plasma	Other	Initials
1	10	—	—	H
2	10	—	—	H
3	10	—	—	H
4	10	—	—	H
5	10	—	—	H
6	10	—	—	H
7	10	—	—	H
8	10	—	—	H
9	10	—	—	H
10	10	—	—	H
11	10	—	—	H
12	10	—	—	H

166

NORTH AM-CCIP Project
Immunization Procedure

SEARLE

INVESTIGATOR: KulROOM NO. 105

DATE: _____

TECH: JT+GMSPECIES: Rabbit

ANIMAL I.D. #	WEIGHT (lbs/kg) <u>g</u>	OTHER
<u>1</u>	<u>1900</u>	
<u>2</u>	<u>1901.1</u>	
<u>3</u>	<u>2070.1</u>	
<u>4</u>	<u>2051.7</u>	
<u>5</u>	<u>2154.3</u>	
<u>6</u>	<u>2145.6</u>	
<u>7</u>	<u>2040</u>	
<u>8</u>	<u>2116</u>	
<u>9</u>	<u>2002.8</u>	
<u>10</u>	<u>1942</u>	
<u>11</u>	<u>1945.4</u>	
<u>12</u>	<u>2084</u>	

Rabbits Initial Weights

Signature

Jim Kul

Date

Read and Understood By

J. Hays

Note

second Immunization -

On weighed out the following: (whole resin)

RABCTP-2	3.5 mg	(2 ^{into} rabbits)
RABCTP-3	4.3	"
" - 4	4.7	"
" - 5	4.0	"
" - 6	3.8	"
" - 7	4.2	"

Dissolved in 1.5 ml sterile PBS, pH 7.4.

According to Butz, S. et al. (1994) Peptide Research 7: 20-23 sonication of beads leads to partial breakage of bead to make it more accessible to immune system.

Solutions of beads were sonicated with nucloph at maximum setting for 2-3 minutes (minimum). Not much change in turbidity of solution - beads still look intact & settle out quickly. Continued to sonicate - each up to 5 minutes with little change. (It is not clear how long the Butz paper authors sonicated their beads).

Left beads in PBS @ 4°C overnight.

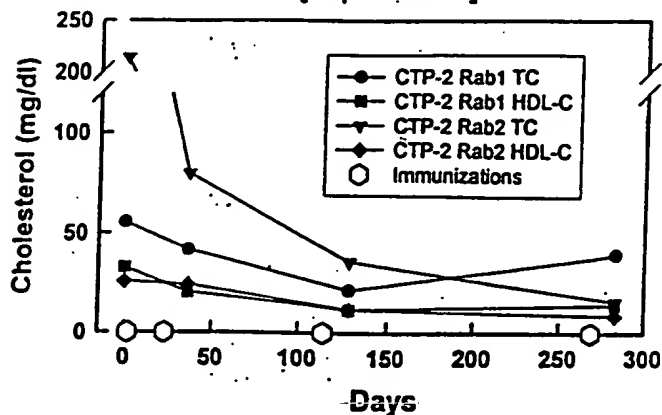
On warmed bead solution to room temperature. Added 1.5 ml INCOMPLETE Freund's Adjuvant (Sigma). Emulsified using 2 x 5cc syringes as before (p. 165).

Immunized rabbit subcutaneously (~1.5 ml of each emulsion) in multiple sites on back.

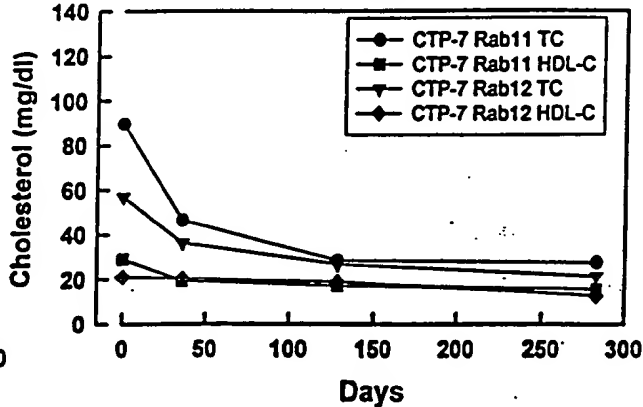
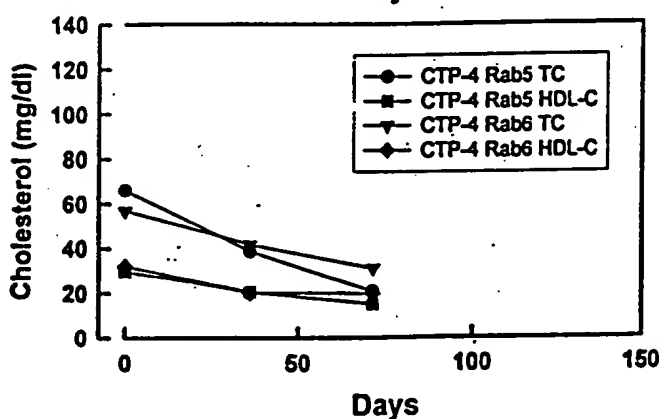
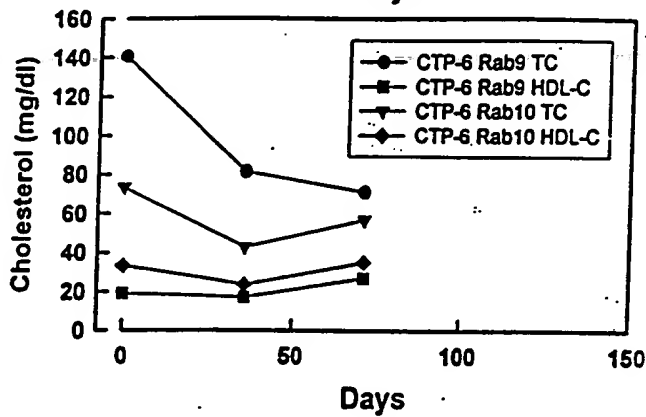
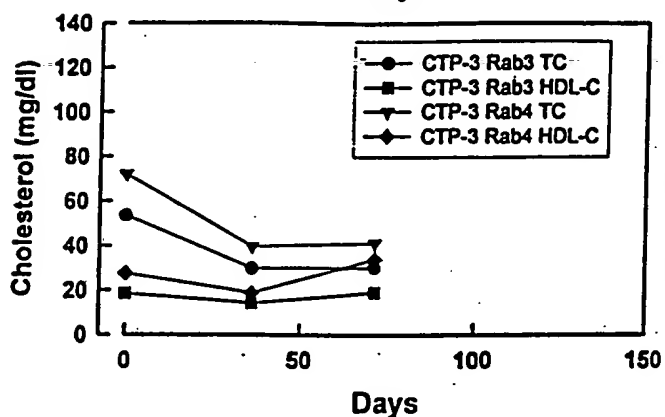
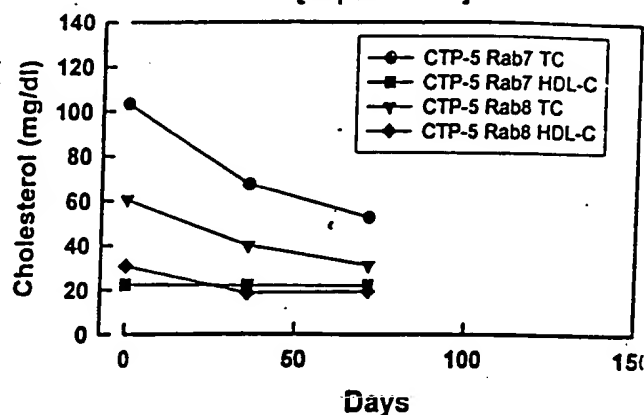
Bloods to be taken on:

Project Number	Subject <i>Summary of Lipid is up on Antisera from CTP-Peptide Immunized Rabbits</i>	Book Number
SEARLE		Page 071

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]



Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]

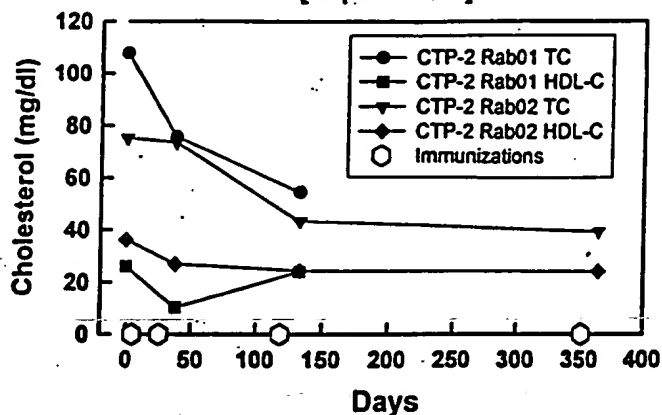


Conclusions: Initial drop in cholesterol may be adaptation to new diet (vendor vs. Searle) or consequence of immunizing per se. Possible effects of CTP-3, CTP-6 immunizations in raising HDL. Curiously no Ab bound to peptide on ELISAs (see 5634011) for

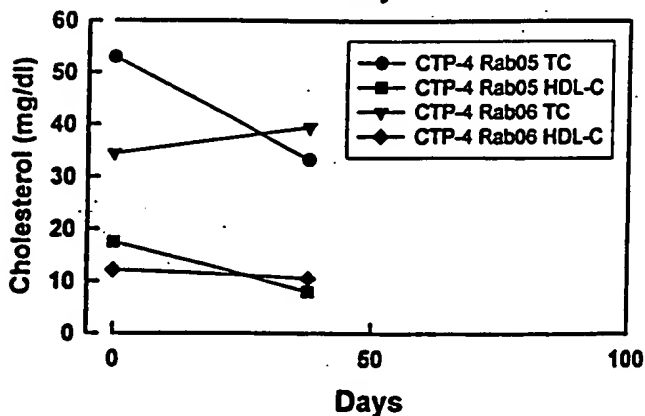
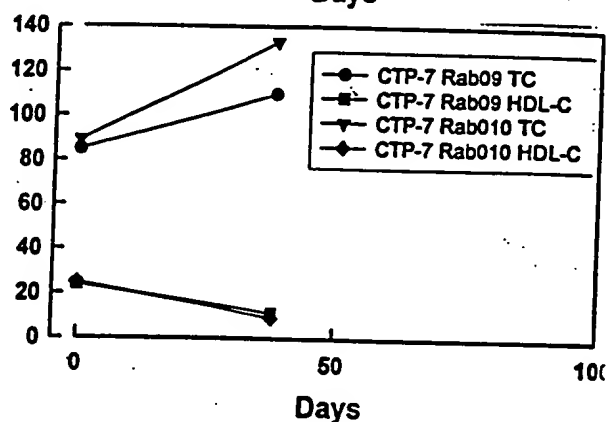
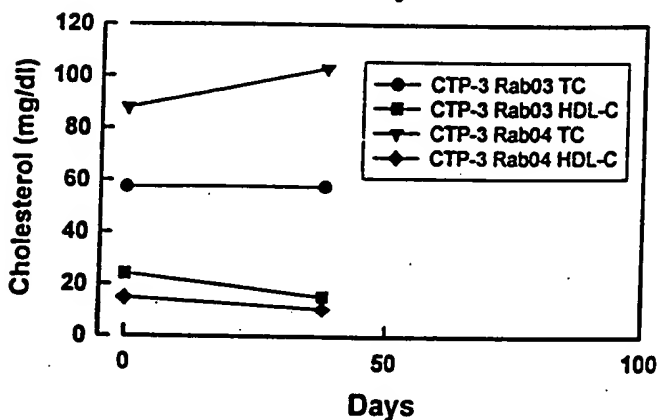
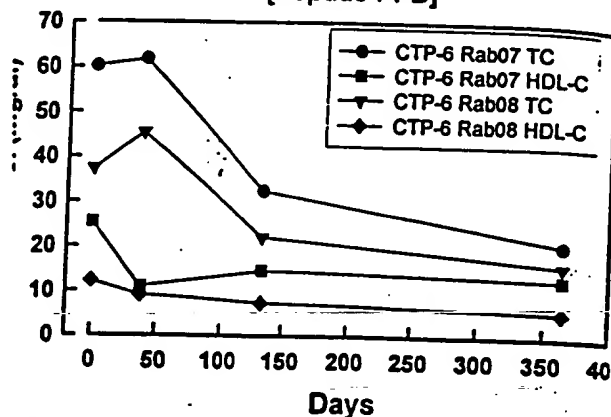
Author's Signature <i>Elaine Kuhl</i>	Date	Read and Understood By <i>Denise Nachowiak</i>
--	------	---

Book Number	Subject	Project Number
Page	Summary of Lipid Assays on Antisera from CTP-Peptide Immunized Rabbits	SEARLE
072		

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]



Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]



*ELISA results
See

Conclusion: Drop in cholesterol seen over time. Not clear why. Only CTP-2 & CTP-6 showed Abs to peptide on ELISA. One rabbit that died (CTP-2) appeared to have HDL raising. Also rabbit 07 (CTP-6) appeared to have HDL elevation. No effect on HDL seen in CTP-3, CTP-4 or CTP-7.

Author's Signature	Date	Read and Understood By	Date
Elaine Krul		Denise Nachowiak	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Needleman et al.)
) Attorney Docket
Serial No.:	08/788,882) 6221/69242
) MON-102.0
Filed:	January 21, 1997)
) Art Group:
For:	AN IMMUNOLOGICAL PROCESS AND) 1642
	CONSTRUCTS FOR INCREASING)
	THE HDL CHOLESTEROL CONCENTRATION)
)
Examiner:	T. Scheiner)

DECLARATION OF PHILIP NEEDLEMAN AND
KEVIN GLENN PURSUANT TO 37 C.F.R. §1.131

Assistant Commissioner for Patents
 Washington, D.C. 20231

Sir:

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Declare:

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 that are the named inventors of the subject patent application;

2. That they are employed by G.D. Searle, Co.,
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 (Monsanto), the assignee of the above-identified patent
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3. That Dr. Needleman holds the positions of Senior
 Vice President of Research and Development at Searle and Chief

*Considered
 45
 9/12/99*

Scientist at Monsanto, whereas Dr. Glenn holds the titles of Fellow and Group Leader of Atherosclerosis Research at Searle;

4. That Elaine Krul, Ph.D., who is presently employed by the Nutrition and Consumer Sector, a division of the Monsanto Company, was formerly employed by Searle between February of 1994 and February of 1998 doing laboratory research related to atherosclerosis;

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13. That twelve rabbits were immunized on two occasions, two each, with a MAP-linked polypeptide conjugate received from Genosys;

14. That those two immunizations were recorded on pages 165, 166 and 167 of her above notebook, copies of which are enclosed as collective Exhibit 4;

15. That the samples of the immunized rabbits' blood were taken thereafter and were assayed for total cholesterol (TC) and high density lipoprotein (HDL-C);

16. That enclosed Exhibit 5 is a copy of page 071 of a second of her notebooks (notebook B) that contains graphs of the total cholesterol (TC) and high density lipoprotein (HDL-C) for each of the rabbits used in the study from prior to the immunizations (day zero) through at least 75 days thereafter, and in which the boxes within each graph identify each peptide with a shortened designation (CTP-number rather than RABCTP-number) because of space considerations;

17. That those data indicate a general lowering of both total cholesterol and high density lipoprotein for each of the peptides used;

18. That total cholesterol was determined in a standard laboratory assay commercially available from Wako Pure

Chemical Industries, Inc., adapted to a 96-well microtiter plate format;

19. That high density lipoprotein was determined in a standard laboratory assay using a commercially available kit from Sigma Chemicals, Inc., of St. Louis, MO;

20. That enclosed Exhibit 6 is a copy of page 072 of her notebook B showing data for rabbits immunized with conjugates of free polypeptides RABCTP-2, -3, -4, -6 and -7 (again referred to as CTP-2, CTP-3 etc.), also obtained from Genosys, that were individually covalently bonded to tuberculin purified protein derivative (PPD) to form conjugates, and prepared as discussed in Example 1B of the above-identified application, beginning at page 37;

21. That the results shown in Exhibit 6 were obtained following two immunizations with the conjugates using the assay procedures discussed in relation to Exhibit 5;

22. That all statements made herein of his (their) own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the


Serial No.: 08/788,882

-6-

United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Enclosures
Exhibits 1-6

2/1/99
Date


Philip Needleman

6 January, 1999
Date


Kevin Glenn

Amber	Subject	Project Number
160	Rabbit anti-CETP Project Choice of Peptides for Immunization	SEARLE

RABBIT CETP SEQUENCE - RABCTP-5 / RABCTP-6 / RABCTP-7

RABCTP-7

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFORAGYPDVSGERAVM

10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNSVVFKGTLNYS

60 70 80 90 100

YTSAWGLQINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110 120 130 140 150

RABCTP-6

LLHLOGEREPGWLKQLETFNFISFTLKLILKGQVCNEINTISNIMADFVQT

160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKHGFTHKNVSEAFPLRAF

210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARAQFQEGRLVLSLTGDEFKKVLETQG

260 270 280 290 300

EDTNQEIQELSRGLPTGQAQVAHVHCLKVPKISCQNRGVVSSSVAVTFR

310 320 330 340 350

RABCTP-5

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCPASGRAGSS

360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSESLSLRS LIATVG IPEVMSRLEVAFT

410 420 430 440 450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLVDFLQSL

460 470 480 490

Project Number	Subject	Book Number
SEARLE	Rabbit Anti-CETP Project Choice of Peptides for Immunization	Page 159

RABBIT CETP SEQUENCE - RABCTP-3 / RABCTP-4

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQIRAGYPDVSGERAVM

10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVEKGTNLNS

60 70 80 90 100

YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110 120 130 140 150

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFEVQT

160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARAQFQEGRLVLSLTGDEFKKVLETQG

260 270 280 290 300

RABCTP-3

EDTNQEIQELSRGLPTGQAQVAHCLKVPKISCQNRGVVSSSVAVTFR

310 320 330 340 350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS

360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSESLQSSLRSLIATVGIPVMSRLEVAFT

410 420 430 440 450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLVLVDFLQSL

460 470 480 490

RABCTP-4

RABBIT CETP SEQUENCE - RABCTP-2

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQAGYPDVSGERAVM

10	20	30	40	50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

60	70	80	90	100

YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAHKL

110	120	130	140	150

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFEVQT

160	170	180	190	200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210	220	230	240	250

PPGLLGDSRMLYFWFSDQVLNSLARAFAEQEGRVLVSLTGDEFKKVLETQG

260	270	280	290	300

FDTNQEIFQELSRGLPTGQAQVAVHCLKVPKISCQNRGVVVSSSVAVTFR

310	320	330	340	350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS

360	370	380	390	400

ANLSVALRTEAKAVSNLTESRSESLQSSLRSLIATVGPIPEVMSRLEVAFT

410	420	430	440	450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDFLQSL

460	470	480	490

Summary of Peptide Sequences Submitted to Genosys to Couple on MAP:

RABCTP-2	306-325	(#001)	Order # 054215 P.O.# 8002455
RABCTP-3	345-364	(#002)	40µM of each made - Immuno
RABCTP-4	475-496	(#003)	Grade (>60% pure)
RABCTP-5	370-389	(#004)	<u>Made on MAPS Resin:</u> See reference: Butz, S. et al. (1994) Peptide Research 7: 20-23.
RABCTP-6	150-169	(#005)	
RABCTP-7	42-61	(#006)	

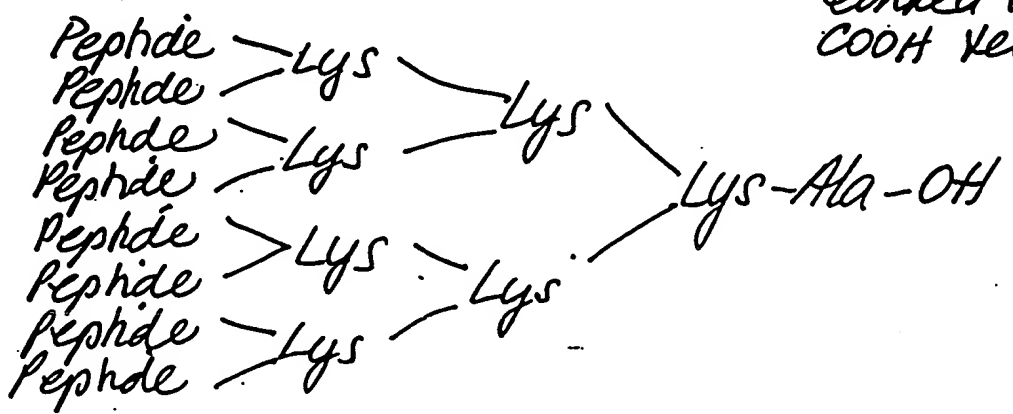
Decided to try coupling to Multiple Antigenic Peptide Backbone as a way to avoid "carrier" protein which will generate a lot of antibodies of and in itself.

One of the original references to this method is:

Posnett, DN, ~~Atkins~~ McGrath H, & Tam JP (1988)
 J. Biol. Chem. 263: 1719-1725.

Structure of MAP:

NOTE: Peptides are linked via their COOH termini!!



GENOSYS
 Genosys Biotechnologies, Inc.
 1442 Lake Front Circle, Suite 185
 The Woodlands, TX 77380-3600
 (713) 363-3693
 FAX (713) 363-2212

Stacey ext #127
Hogue

[615-343-1465]
James Tam

0.37mm/g
0.37µm/mg rem

Dr. Stanfield
 Page 1
713-363-3693 ext 13:

Order No.

54215

ORDER

Bill To
 MONSANTO COMPANY
 ACCOUNTS PAYABLE. N2F
 800 NORTH LINDBERGH BLVD.

Ship To
 DR. ELAINE KRULL
 MONSANTO COMPANY
 60-000-760.92
 T213W/T2M
 800 N. LINDBERGH BLVD
 ST. LOUIS

ST. LOUIS

MO 63167

MO 63167

---CONTACT---
 BARB GRIFFARD
 314-694-1000 X 6825

---CONTACT---
 314-694-4218

Customer: 100 6316701 KRUI
 Sal sman: 8
 Terms: NET 30
 SHIP VIA: Airborne (Prepaid)
 /O Number: B002455

ORDERED:
 SHIPPED:
 INVOICED:

QTY	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
1.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 µm IMMUNO GRADE RABCTP-2 EIF QEL SRG LPT GQA QYA VH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00			~87mg
									950
2.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 µm IMMUNO GRADE RABCTP-3 YAV TFR FPR PDG REA YAY RF RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00			~94mg
									950
3.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 µm IMMUNO GRADE RABCTP-4 LLL QND FGF PKH LLV DFL QSL S RESIDUES IMMUNO GRADE MAPS RESIN PEP SYN	22.00	1.030			~102mg
									950
4.000	1.00	0.00	PEPTIG/40	CUSTOM PEPTIDE 40 µm IMMUNO GRADE RABCTP-5 TTY QAS YSQ KKL FLH LLO FO RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00			~95mg
									950

MW 2181

Lot P157A-022
peptide # 54215-001

Lot P157A-024

MW 2355
peptide # 54215-002

Lot # P157A-026

MW 2562
peptide # 54215-003

MW 2368
Lot P157A-028
peptide # 54215-004

ACKING LIST

T I N U E

GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Order No.

Page 2

Bill T
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

ST. LOUIS MO 63167

54215

ORDER

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.
ST. LOUIS MO 63167

CONTACT
BARB GRIFFARD
314-694-1000 X 6825

CONTACT
314-694-4218

Customer: 100 6316701 KRUI
Salesman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
P/O Number: B002455

ORDERED:

SHIPPED:
INVOICED:

THE	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
5.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-6 LLL HLG GER EPG NLK QLF TH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	
MW 2393 + P157A-030 lephide 54215-005				~96mg					
6.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-7 DVS GER AVH LLG RVK YGL HH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	
MW 2215 + P157A-032 lephide 54215-006				~89mg					

COMMENTS:
cleaned by 95% TFA, ether extracted 3X, suspended in
0.1M TFA/H₂O - 1-2% acetic acid resuspended
+ lyophilized
↓
lyophilized

MISCELLANEOUS

FREIGHT

4.624.00

TAX

TOTAL

PACKING LIST

Project Number:	Subject: <u>Rabbit anti-CETP Project</u>	Book Number:
SEARLE	<u>Immunization Procedure</u>	Page: 165

Immunization of Rabbits

Subcutaneously administered (0.05-0.1 ml/site)
along rabbit's back - done by Margi Baldwin.

Rabbits in Room 105 (Tattooed)

#1, #2	RABCTP-2	(3.3mg total ÷ 2 rabbits))
#3, #4	RABCTP-3	(3.4mg "))
#5, #6	RABCTP-4	(3.2mg "))
#7, #8	RABCTP-5	(3.2mg "))
#9, #10	RABCTP-6	(3.3mg "))
#11, #12	RABCTP-7	(3.5mg "))

INVESTIGATOR: _____ DATE: _____
 room #: _____ phone: # _____ Mail Zone: Tam
 Technician: _____

RE-
immune
era
bleed

red
pen in
ignob
-200C.

Animal ID #	Bleed	Serum/Plasma	Other	Initials
1	10	_____	_____	H
2	10	_____	_____	Q
3	10	_____	_____	H
4	10	_____	_____	H
5	10	_____	_____	H
6	10	_____	_____	H
7	10	_____	_____	H
8	10	_____	_____	H
9	10	_____	_____	H
10	10	_____	_____	H
11	10	_____	_____	H
12	10	_____	_____	H

INVESTIGATOR: KuhlROOM NO. 105

DATE: _____

TECH: JT + CMSPECIES: Rabbit

ANIMAL I.D. #	WEIGHT (g) ^g	OTHER
<u>1</u>	<u>1900</u>	_____
<u>2</u>	<u>1901.1</u>	_____
<u>3</u>	<u>2070.1</u>	_____
<u>4</u>	<u>2051.7</u>	_____
<u>5</u>	<u>2154.3</u>	_____
<u>6</u>	<u>2145.6</u>	_____
<u>7</u>	<u>2040</u>	_____
<u>8</u>	<u>2116</u>	_____
<u>9</u>	<u>2002.8</u>	_____
<u>10</u>	<u>1942</u>	_____
<u>11</u>	<u>1965.4</u>	_____
<u>12</u>	<u>2084</u>	_____

Rabbit's Initial Weights

SEARLE

2nd Immunization -

On weighed out the following: (whole resin)

RABCTP-2	3.5 mg	(2 ^{into} rabbits)
RABCTP-3	4.3	"
" - 4	4.7	"
" - 5	4.0	"
" - 6	3.8	"
" - 7	4.2	"

Dissolved in 1.5 ml sterile PBS, pH 7.4.

According to Butz, S. et al. (1994) *Peptide Research* 7: 20-23
sonication of beads leads to partial breakage of
bead to make it more accessible to immune
system.

Solutions of beads were sonicated with nuclohp at
maximum setting for 2-3 minutes (minimum). Not
much change in turbidity of solution - beads still
look intact & settle out quickly. Continued to
sonicate - each up to 5 minutes with little
change. (It is not clear how long the Butz paper
authors sonicated their beads).

Left beads in PBS @ 4°C overnight.

In warmed bead solution to room temperature.
Added 1.5 ml INCOMPLETE Freund's Adjuvant (Sigma).
Emulsified using 2 x 5cc syringes as before (p. 165).

Immunized rabbit subcutaneously (~1.5 ml of each
emulsion) in multiple sites on back.

Bleeds to be taken on:

Signature

Zaine Krul

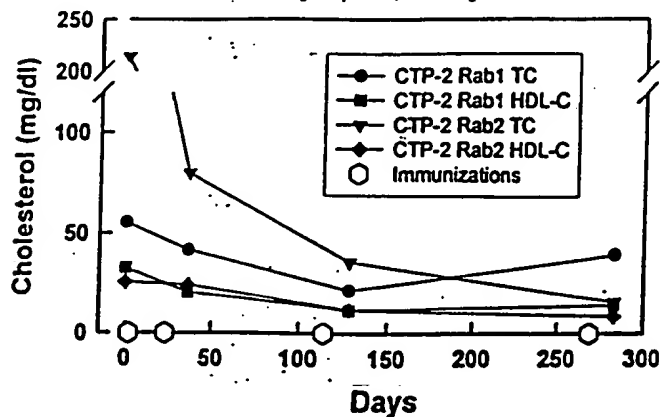
Date

Read and Understood By

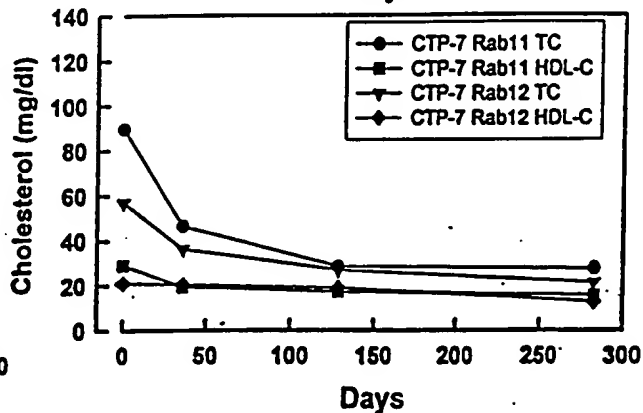
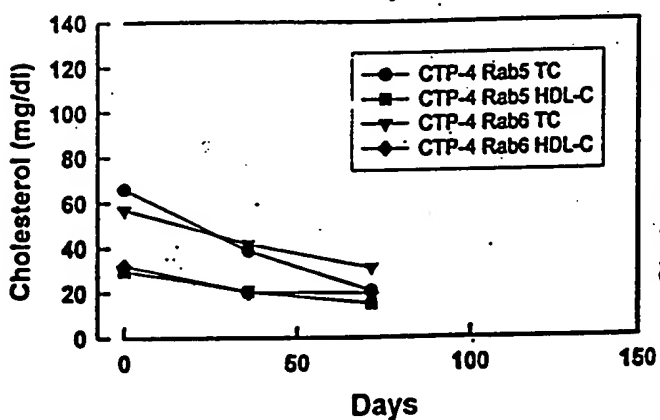
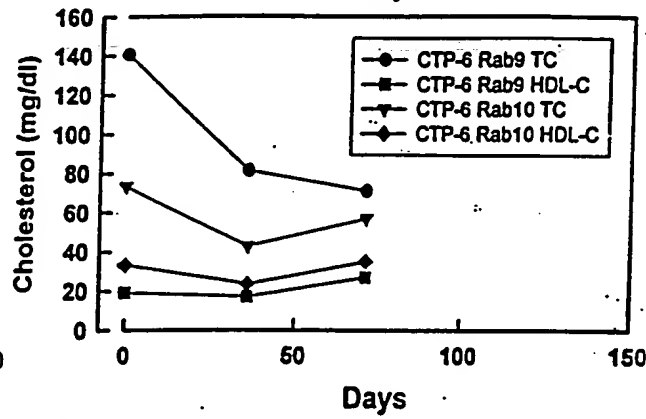
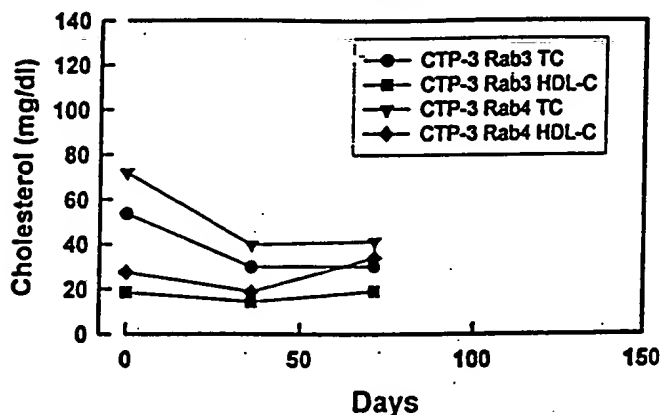
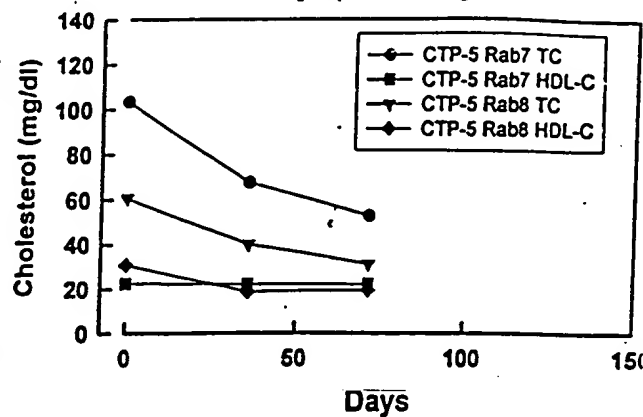
S. Kan

Date

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]

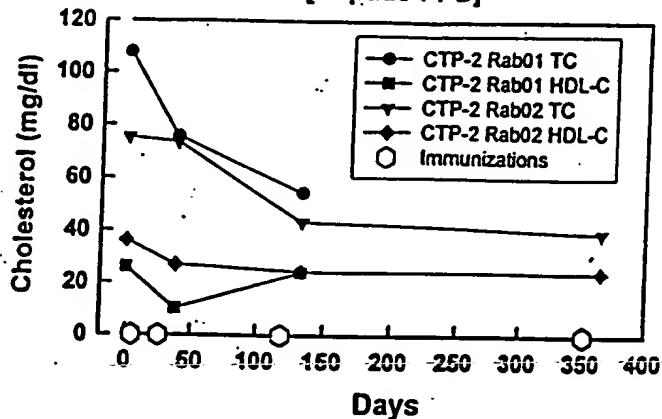


Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]

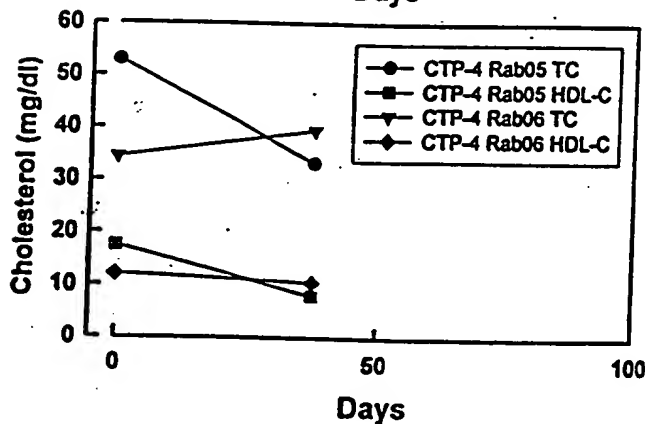
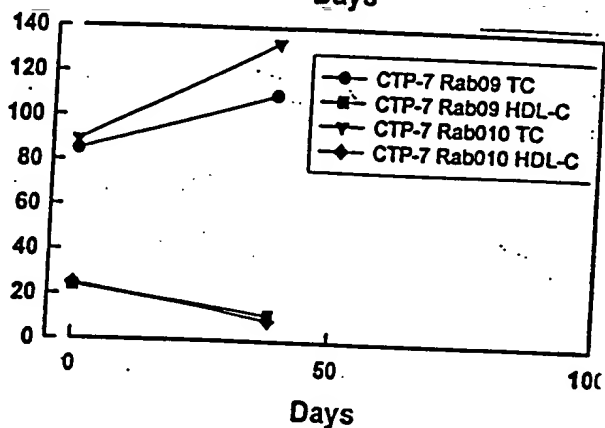
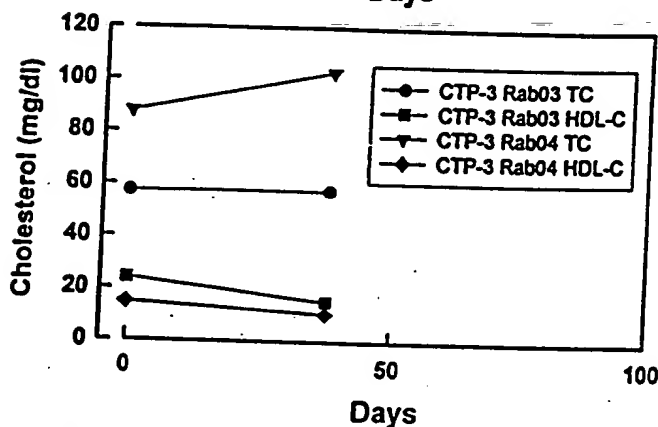
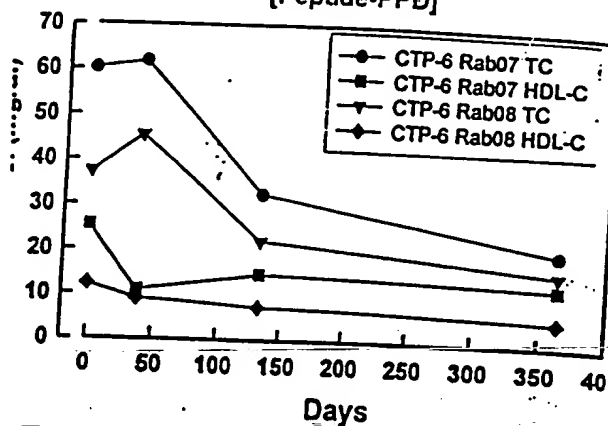


Conclusions: Initial drop in cholesterol may be adaptation to new diet (Vendor vs. Searle) or consequence of immunizing per se. Possible effects of CTP-3, CTP-6 immunizations in raising HDL. Curiously no Ab bound to peptide on ELISAs (see 5634011) for

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]



Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]



*ELISA results
See

Conclusion: Drop in cholesterol seen over time. Not clear why only CTP-2 & CTP-6 showed Abs to peptide on ELISA. *One rabbit that died (CTP-2) appeared to have HDL raising. Also rabbit 07 (CTP-6) appeared to have HDL elevation. No effect on HDL seen in CTP-3, CTP-4 or CTP-7.